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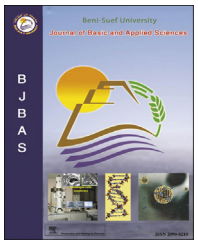

Full Length Article

Therapeutic potential of *Garcinia kola* with reference to the restoration of inhibited acetylcholinesterase activities in induced *Clarias gariepinus*

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ABSTRACT

This study was conducted to assess the antidotal prospect of *Garcinia kola* seeds extract in restoring the activity of inhibited Acetylcholinesterase. This was done by inducing *Clarias gariepinus* with the enzyme inhibitor (glyphosate pesticide formulation). The fish divided into six groups were exposed different treatments; the pesticide alone, the *G. kola* seed extract alone and different mixture of the pesticide and *G. kola* seeds extract. AChE activities in the brain, liver and serum of the fish were measured in the experimental and control fish on day −7, 14, 21 and 28th by the colorimetric method. The enzyme was significantly ($p < 0.05$) inhibited in the glyphosate formulation test alone and in group IV treatment (0.16 mg/L glyphosate formulation with 150 mg/L of extract). The inhibition percentages of AChE ranged for the brain, liver and serum between 40.7–59.4%, 50–57% and 27.5–51.3%, respectively. The AChE activities were however, recovered in *G. kola* seeds extract treated aquaria, and were dose, time dependent and organ specific. Modifications of this enzyme may leads to increased perspiration, increased salivation, tearing, blurred vision, abdominal cramping, diarrhea, and if severe enough, death from respiratory depression. This investigation had revealed the therapeutic significance of *G. kola* seeds extract, by stabilizing the enzyme activity in the investigated fish. Further investigation is required to measure the concentrations of acetylcholine at cholinergic synaptic junction in fish and mammals induced with ant-cholinesterase agent and the possibility of its restoration using *G. kola* seeds.

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1. Introduction

Acetylcholinesterase (AChE) is one of many important enzymes needed for the proper functioning of the nervous systems of humans, fish and insects (Carlock et al., 1999; Ezemonye and Ikpesu, 2012). The enzyme catalyzes the hydrolysis of acetylcholine to choline and acetyl CoA, which are reabsorbed and used as raw materials for the continued acetylcholine production. Its inhibition results in a buildup of

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acetylcholine at sites of cholinergic synapses and lead to excessive stimulation of the nerve/muscle fibers without control until spasm, paralysis, convulsion and death (Galloway and Handy, 2003).

The enzyme activity can be easily disrupted by contaminants such as organophosphate and carbamate pesticides. These pesticides disrupt nerve impulse transmission in the central and peripheral nervous system by inhibiting AChE, located at neuromuscular junctions and in plasma or haemolymph of mammalian red blood cells (Galloway and Handy, 2003).

Manny refined drugs have been successfully used in restoring the enzyme activity; however using plant products such as *Garcinia kola* has not been seriously investigated. Medicinal plants play a key role in the slowing of many pathogenesis and neurodegenerative disorders such as Alzheimer and dementia (Rehman, 1995; Dai et al., 2006). Galantamine, an Amaryllidaceae alkaloid obtained from *Galanthus nivalis* was used traditionally in Bulgaria and Turkey for the treatments of neurological conditions (Lopez et al., 2002). The lycopodium alkaloid huperzine related to the quinolizidines, is a potent, yet reversible, inhibitor of AChE and is used in China for treating patients with myasthenia gravis (Lopez et al., 2002). Similarly, *Oligomer viniferin* from Caragana chamlague, has also been identified as reversible and non-competitive inhibitor of AChE (Da-Yuan et al., 1996). Daily consumption of fresh vegetable reported in delaying of cognitive decline in older age (Letenneur et al., 2007). Beneficial effects of these metabolites are proposed to associate with potentiating of antioxidant defenses, which is linked to normal aging and neurodegenerative diseases (Morris et al., 2006). Similarly, *Arnica montana* roots which contain derivatives of thymol, are used as fungicide and preservatives and have anti-inflammatory effect (Weremczuk -Jezyna et al., 2006) and when usedtopically in agel at 50% concentration, the plant was found to have the same effect when compared to a 5%ibuprofen gel for treating the symptoms of hand osteoarthritis (Braga et al., 2006).

G. kola is a medicinal plant that provided raw material for innovative, useful and promising antidote to environmental glyphosate and other xenobiotics in fish and other aquatics contamination (Ikpesu et al., 2014). The plant which belong to the family Guttiferaceae (Heckel and Schlagdenhauffen, 1884) is known to contain high content of bioflavonoid compounds (Iwu, 1986) with a general anecdotal effect in folk medicine in Africa (Adaramoye et al.,2005). Its constituents include- Flavinoids (bioflavonoid), xanthenes and benzophenones and have shown anti-inflammatory, ant parasitic, antimicrobial, antiviral properties (Iwu et al., 1987). Food does not affect the metabolism of *G. kola* and may buffer effects of mild indigestion (Iwu, 1986). The kola is primarily carried bound to albumin in the blood and only a minor amount is metabolized by hepatic metabolism (Iwalewa et al., 2005). It is safe taken with or without other foods. Taking it an hour before or after meals may help to increase the absorption of the key ingredients (Iwalewa et al., 2005). In this investigation, I study the AChE activities in the brain, liver and serum of *C. gariepinus* induced with glyphosate formulation. The fish was chosen for this investigation because of the great geographical distribution of the fish throughout the world (Idodo, 2003), while the pesticide is one of the main inhibitor of AChE.

Sub-lethal effects of pesticides on AChE activity in *C. gariepinus* and other aquatic organisms have been extensively studied. However, the possibility of restoring the enzyme activity has not been seriously investigated. Therefore, the objectives of this investigation are; (i) to determine the activities of the enzyme in the brain, liver and the serum of *C. gariepinus* induced with glyphosate formulation, an anti-cholinesterase agent and (ii) to determine the therapeutic of *G. kola* seeds extract in restoration of the inhibited AChE activities in the fish's tissues.

2. Materials and methods

2.1. *Chemical analysis*

Glyphosate (99.5% purity) and methanol (analytical grade) for high-performance liquid chromatography (HPLC) were obtained from Chemical Service (West Chester, PA, USA). Na_2SO_4 (99% purity), petroleum ether (analytical grade), acetonitrile (analytical grade), Ethyl 3-aminobenzoate methanesulfonate salt (Sigma–Aldrich,USA. High purity pesticide grade solvents (hexane, dichloromethane and the surrogate standard solution) were obtained from Merck (Darmstadt, Germany), helium (purity 99.999%) by Messer Technogas (Czech Republic) and Bovine serum albumin (BSA) used for the determination of protein quantity was purchased from Sigma Chemical Company St Louis, MO, USA.

2.2. *Equipment*

Equipment included heparinized syringe, glassware, Cecil HPLC system comprised of CE 1200 high performance variable wavelength monitor and CEII00 liquid chromatography pump, high-resolution gas chromatography (HRGC), UV detector with variable wavelength and stainless steel column (C18 Reverse phase) packed with Octasilica, vacuum pump, and ultrasonic check.

Test Organisms: One hundred and fifty healthy juveniles of *C. gariepinus* of mean weight (30.00 + 0.13 g) and length (13.09 + 0.2 cm) from fresh water environment were collected from a private farm (Patiby Agro Industrial Enterprise) from Erawa Owhe, Delta State, Nigeria. Fish were examined for any pathological symptoms and acclimatized to laboratory conditions in holding glass tanks containing deionized water for two weeks before they were used for the experiments. The holding tanks were aerated with the help of air pump, cleaned and water renewed daily. Fish were fed on 30% protein pellets, unconsumed feed and faecal wastes were removed and water replenished regularly as recommended by Oyelese and Faturoti (1995).

Collection and preparation of plant extracts: Matured *G. kola* seeds were obtained from a private farm at Walode, Osun State Nigeria. Brown coated seeds were manually removed from the pod and air dried for five days. The dried brown coat was hand peeled, and the seeds cut into pieces and re-dried at room temperature (22 + 0.15)°C for three months (Onunkwo et al., 2004). The seeds sample were grounded using Nakai

blender (dry mill) and filter through a 40-mesh screen. One hundred gram (100 g) of the powdered *G. kola* was spiked with a solution of surrogate standard (d8-naphthalene, d10-acenaphthene, d12-chrysene and d12-perylene) and extracted with a mixture of dichloromethane and n – hexane in a ratio 2: 3, after subjected to a vigorous shaking in a sonication bath for 5 h. The solvent was separated, concentrated using a rotatory evaporator and eluted with methanol. The elute was transferred into an open 250 ml conical flask in a placid environment for 48 h to evaporate the methanol.

2.3. Experimental design

The concentrations of glyphosate formulation for the test were prepared from the stock solution via serial dilution. Stock solution, test water concentrations and *G. kola* seed extract concentration were verified by Cecil HPLC system comprised of CE 1200 high performance variable wavelength monitor and CEII00 liquid chromatography pump and high-resolution gas chromatography (HRGC), using a Hewlett–Packard 5890 capillary gas chromatograph (Hewlett–Packard, Avondale, PA, USA) equipped with an electron capture detector (Hewlett–Packard).

2.4. Acute test

The 96 h LC_{50} of glyphosate formulation for *C. gariepinus* was conducted according to Organization for Economic Cooperation and Development (OECD) Guideline No. 203 for static-renewal test conditions (OECD, 1992). Fifteen glass aquaria were used for this test, with three replicates per treatments. Each aquarium contains different concentrations of the toxicant. All experiments were conducted at room temperature and the tanks were properly aerated. Fish were not fed during the experiment (Reish and Oshida, 1986). Thirty minutes after the preparation of test solution, five juveniles were carefully placed into each tank with their replicate tanks of five different concentrations including the control (0.00, 0.50, 1.50, 2.50 and 3.50 mg/L). Seventy-five percent of the test solution was renewed each day and aerated with the aid of air pump. Fish and water quality parameters (pH, temperature, Dissolved Oxygen (DO), turbidity, alkalinity and total hardness) of the test solution were determined at 24 h interval, using standard methods. Cumulative fish mortality was recorded at 24-, 48-, 72-, and 96-h time intervals and the LC_{50}s of each period calculated using the lethal computer by Finney (1971). Experiment lasted for 96 h for the different concentrations of the pesticide.

2.5. Chronic test

The chronic test was conducted under OECD test guideline 407 (OECD, 1997a). The 96 h LC_{50} of glyphosate formulation for *C. gariepinus* was 2.8 mg/L, evaluated by Probit method (Finney, 1971). One-tenth of the LC_{50} value was selected as a sub-lethal dose. The fish were divided into six groups comprising five -fish each and treated as stated below;

The 1st group was given distilled water as contained in the experimental doses
The 2nd group was treated with 0.16 mg/L glyphosate formulation only
The 3rd group was treated with 350 mg/L of extract of *G. kola* seeds
The 4th group was treated with 0.16 mg/L glyphosate formulation with 150 mg/L of extract of *G. kola* seeds
The 5th group: was treated with 0.16 mg/L glyphosate formulation with 250 mg/L of extract of *G. kola* seeds
The 6th group: was treated with 0.16 mg/L glyphosate formulation with 350 mg/L of extract of *G. kola* seeds

Eighteen glass aquaria were used with 3 replicates per treatments. The water quality parameters (pH, Temperature, DO, Turbidity, Alkalinity, and Hardness) of the test solution were monitored throughout the duration of the experiment. Samples were collected on day 7, 14, 21 and 28th. After each stipulated time, fish were removed from aquaria, decapitated and blood samples taken by puncturing the caudal vessels with a 20-gauge needle and aspirating 0.2–0.4 ml sample of mixed arterial and venous blood into a heparinized syringe, a technique shown to minimize dilution by tissue fluids (Onunkwo et al., 2004). Blood samples were collected in small vials, left to clot and then centrifuged at 3000 rpm for 10 min to obtain serum that was frozen at −80 °C. Brains and livers tissues were isolated through dissection and homogenate in cold 0.15 M Kcl solution using homogenizer. Tissue homogenates were centrifuged at 15,000 rpm for 10 min and used for the enzyme assay.

2.6. AChE assay

The activities of AChE was measured in the supernatant using commercial kit produced by Bohringer Mannhim as based on Ellman spectrophotometric assay procedure (Ellman et al., 1961) adapted for micro plates as described by Handy et al. (2003) using acetylthiocholine iodide as a substrate. Fifty micro liters (50 μl) of the samples in phosphate buffer (pH 7.4) was reacted with 150 μl of DTNB (3 mM) and acetylthiocholine iodide (15 mM). The mixture was incubated at 25 °C for 15 min in microtitre plates and the absorbance measured spectrophotometrically at 412 nm. Enzymatic activities were expressed as units of activity (u) per mg of protein. Each unit of activity corresponded to 1 nmol of substrate hydrolyzed per minute.

2.7. Statistical analysis

Data were analyzed using the Minitab Statistical Computing System (Ryan et al., 1981), SAS (SAS institute Inc, 1985), SPSS version 14.0 (Chicago IL, USA) and Microsoft Excel 2007 (Roselle, IL, USA) were used for the statistical and graphical valuations. The least significant difference (LSD) was determined using post hoc testing for inter group comparisons at a probability level of 0.05% and 0.01%.

3. Results

3.1. Physicochemical properties of the test media

The water quality parameters (pH, temperature, DO, turbidity, alkalinity and hardness) monitored during the exposure

Table 1 – Concentrations of physiochemical parameters of the test media (acute test) of *C. gariepinus* exposed to different concentrations of glyphosate formulation.

Parameters	pH	Temp (°C)	DO (mg/l)	Turbidity(mg/L)	Alkalinity (mg/L)	Hardness (mg/L)
Con (mg/L)	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.000	7.22 ± 0.02	25.67 ± 0.16	8.10 ± 0.22	0.23 ± 0.04	17.40 ± 0.72	31.23 ± 1.15
0.50	7.36 ± 0.16	25.00 ± 0.30	8.12 ± 0.19	0.23 ± 0.06	17.63 ± 0.42	31.33 ± 1.15
1.50	7.25 ± 0.10	26.33 ± 0.68	8.30 ± 0.31	0.24 ± 0.02	18.40 ± 0.36	31.20 ± 1.02
2.50	7.12 ± 0.25	26.00 ± 1.20	8.03 ± 0.22	0.25 ± 0.04	17.13 ± 1.20	30.60 ± 0.50
3.50	7.12 ± 0.20	26.33 ± 0.48	8.16 ± 0.02	0.26 ± 0.03	18.23 ± 0.12	30.60 ± 0.16

periods (acute and chronic tests) were not significantly different between various treatments and the control and within different treatments ($p > 0.05$). Water temperature remained between 25.00 ± 0.30 and 26.33 ± 0.68 °C; pH between 6.82 ± 1.11 and 7.36 ± 0.16, alkalinity 17.00 ± 1.20 and18.40 ± 0.30, harness; 30.00 ± 0.21 and 31.40 ± 1.10, turbidity 0.20 ± 0.02 and 0.26 ± 0.03 and the rate of DO was kept between 7.90 ± 0.12 and 8.16 ± 0.02, with the help of an air pump (Tables 1 and 2).

3.2. *Acute toxicity*

Mortality increased with increasing concentrations of glyphosate formulation in the treated fish. No death was recorded in the control group during this study. Abnormal behaviors such as erratic swimming, coughing, increased movement, physical disorientation, partial jerking, Whip-like behavior, a comparatively longer span in respiratory deficiency or discomfort in fish were observed in induced fish. At the highest concentration, the fish were sluggish and remained at the bottom calmly, which was apparent on the last day of the bioassay test. The 96 h of LC_{50} value for *C. gariepinus* was 2.8 mg/L. Mean percentage mortality of the fish was significantly affected by the pesticide ($p < 0.05$).

3.3. *Chronic toxicity*

Brain: The activities of AChE in the brain of *C. gariepinus* exposed to different concentration of glyphosate formulation, *G. kola* seeds extract, and mixture glyphosate formulation and *G. kola* seeds extract is shown in Fig. 1, with further illustration in Appendix 1. The inhibition was significant (0 < 0.05) between the control and the glyphosate formulation treatments alone and between the control and the group 4 treatments. The percentage inhibition increases with increased with exposure duration. While in *G. kola* seeds extract treatments alone, the enzyme activities was comparable with the control and was not significant ($p > 0.05$) (Fig. 1). The activities of the enzyme in *G. kola* treatments groups (5–6) revealed dose and time dependent recovery and was restored on day 14th

Liver: The AChE activity in the fish's liver exposed to different treatments is shown in Fig. 2 with further illustration in Appendix 1. The inhibition varies significantly ($p < 0.05$) between the control and glyphosate formulation treatments alone and between the control and group 4 treatments. The highest and the lowest percentage inhibition in glyphosate formulation treatments were 57.2% and 43.8% and was recorded on day 7th and 28th respectively. The activities of the AChE in *G. kola* treatments 5th and 6th were restored on day 28th, no effects was recorded in *G. kola* seeds extract treatments alone.

Serum: The activity of AChE in the serum of *C. gariepinus* subjected to various treatments of glypohosate formulation and *G. kola* seed extract is shown in Fig. 3, with further illustration in Appendix 1.The inhibition varies significantly (0 < 0.05) between group 1 and 2, and between group 1 and 4 only. The percentage inhibition in group 2 treatments on day 7, 14, 21 and 28th were 27.5, 38.7, 50 and 51.3 respectively. No inhibition of the enzyme activities observed on day 28th in group 4, while in group 5, inhibition stops on day 21st

Table 2 – Concentrations of physiochemical parameters of the test media (chronic test) of *C. gariepinus* exposed to different of glyphosate formulation and *G. kola seeds* extract.

Parameters	pH	Temp (°C)	DO (mg/l)	Turbidity(mg/L)	Alkalinity (mg/L)	Hardness (mg/L)
Groups	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
1	6.82 ± 0.10	24.78 ± 0.20	7.90 ± 0.12	0.20 ± 0.04	18.00 ± 0.72	30.20 ± 2.15
2	7.06 ± 0.12	25.20 ± 0.20	8.00 ± 0.10	0.25 ± 0.06	17.60 ± 0.42	30.12 ± 0.15
3	6.85 ± 0.11	25.30 ± 0.20	8.00 ± 0.20	0.20 ± 0.02	18.10 ± 0.36	30.19 ± 1.13
4	7.02 ± 0.15	26.30 ± 0.25	7.93 ± 0.04	0.23 ± 0.06	17.00 ± 1.20	31.32 ± 1.50
5	7.00 ± 0.16	25.83 ± 0.30	8.20 ± 0.14	0.22 ± 0.03	17.63 ± 0.12	31.40 ± 1.10
6	6.90 ± 0.12	26.00 ± 0.10	7.90 ± 0.10	0.20 ± 0.02	18.30 ± 0.10	30.00 ± 0.21

Group 1: De-chlorinated water only.
Group 2: 0.16 mg/L glyphosate formulation only.
Group 3: 350 mg/L of extract of *G. kola* seeds.
Group 4: 0.16 mg/L glyphosate formulation and 150 mg/L *G. kola* seeds extract.
Group 5: 0.16 mg/L glyphosate formulation and 250 mg/L *G. kola* seeds extract.
Group 6: 0.16 mg/L glyphosate formulation and 350 mg/L *G. kola* seeds extract.

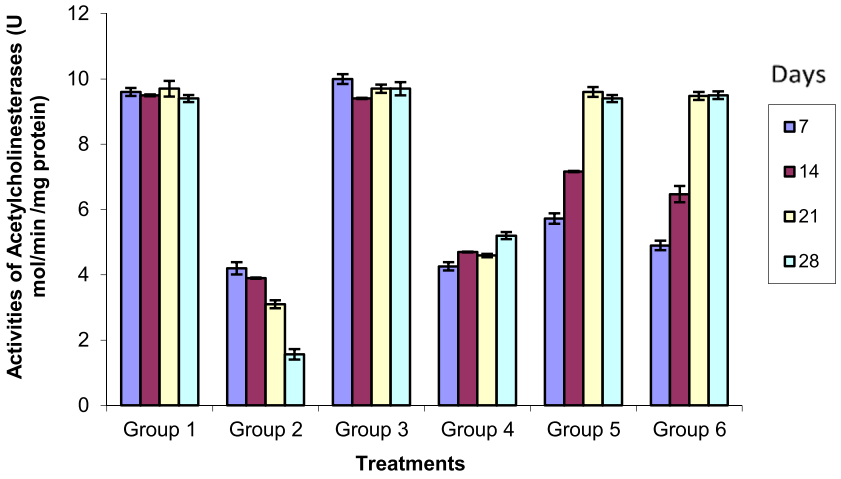

Fig. 1 – Activities of Acetylcholinesterase (U mol/min/mg protein) in the brain of *C. gariepinus* exposed to different treatments on day 7,14,21 and 28th. Group 1: De-chlorinated water only; Group 2:0.16 mg/L glyphosate formulation only; Group 3: 350 mg/L of extract of *G. kola* seeds; Group 4: 0.16 mg/L glyphosate formulation and 150 mg/L *G. kola* seeds extract; Group 5: 0.16 mg/L glyphosate formulation and 250 mg/L *G. kola* seeds extract; Group 6: 0.16 mg/L glyphosate formulation and 350 mg/L *G. kola* seeds extract.

4. Discussion

4.1. *Water quality parameters*

The physicochemical parameters were almost uniform all through the study irrespective of the treatments, duration and the organisms and were within the desirable range of fish culture (FEPA, 1991; Campbell, 1978; WHO, 2006). The non significant changes in the water parameters of various experimental media reported in this study showed that the acute and sub lethal concentrations of glyphosate formulation did not adversely lead to reduction in water quality where slight changes were observed.

4.2. *Mortality*

No death and no morphological changes were observed in the control and chronic toxicity test. This indicates that the mortality recorded in the acute test was as a result of the concentration of the pesticide. Prior to mortality, there was uncoordinated movement with reduced activity evidenced by vertical positioning, curling of spine and vertical movement of the fish, which may probably be due to the loss of equilibrium. All these observations were more pronounced with increasing concentrations of toxicant. Consequently, the percentage and number of survivors decreased with increasing concentrations of toxicants in water. The mortality pattern may be attributed to the injuries on the skin caused by progressive

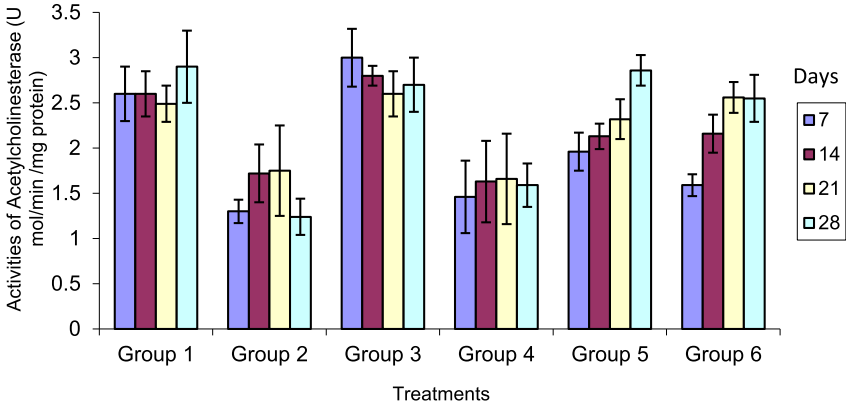

Fig. 2 – Activities of Acetylcholinesterase (U mol/min/mg protein) in the liver of *C. gariepinus* exposed to different treatments on day 7,14,21 and 28th. Group 1: De-chlorinated water only; Group 2:0.16 mg/L glyphosate formulation only; Group 3: 350 mg/L of extract of *G. kola* seeds; Group 4: 0.16 mg/L glyphosate formulation and 150 mg/L *G. kola* seeds extract; Group 5: 0.16 mg/L glyphosate formulation and 250 mg/L *G. kola* seeds extract; Group 6: 0.16 mg/L glyphosate formulation and 350 mg/L *G. kola* seeds extract.

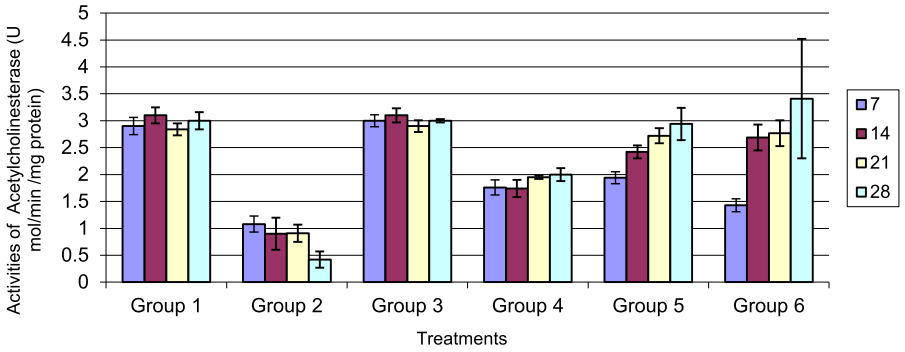

Fig. 3 – Activities of Acetylcholinesterase (U mol/min/mg protein) in the serum of *C. gariepinus* exposed to different treatments on day 7,14,21 and 28th. Group 1: De-chlorinated water only; Group 2:0.16 mg/L glyphosate formulation only; Group 3: 350 mg/L of extract of *G. kola* seeds; Group 4: 0.16 mg/L glyphosate formulation and 150 mg/L *G. kola* seeds extract; Group 5: 0.16 mg/L glyphosate formulation and 250 mg/L *G. kola* seeds extract; Group 6: 0.16 mg/L glyphosate formulation and 350 mg/L *G. kola* seeds extract.

exposure to toxicants which is capable of weakening the organism's resistance and the immune system. Fish mortality was found to significantly increase ($p < 0.05$) with exposure duration and increase in concentration. High mortality recorded in tests with the highest concentrations of 2.50 and 3.50 mg/L within short term of exposure may be due to increased energy demand of detoxification processes as described by Wiegand et al. (1999).

AChE is an important enzymes needed for the proper functioning of the nervous systems of vertebrates and insects. Therefore, measurement of its activities in tissues and organs has been recognized as the standard procedure for post mortem diagnosis of lethal poisoning by anti-cholinesterase chemicals (Walujo and Frederick, 1991). Luke et al. (1975) reported that 20% inhibition of brain AChE indicated exposure to chemicals such as pesticide, while inhibition of ≥50% indicated death from poisonous substances. The effects of cholinesterase inhibition include but not limited; bradycardia, hypotension, hypersecretion, broncho constriction, gastro intestinal tract hypermotility and decrease intraocular pressure and actions on the neuromuscular junction will result in prolonged muscle contraction.

The toxicity of organophophate and carbamate pesticides toward AChE has been extensively studied both in insects and vertebrates (Haubruge and Toutant, 1997; Bocquene et al., 1995), however, antidote prospect using therapeutic has not been seriously investigated in fishes and other aquatic organisms except in natural condition where the insecticides was discontinuous (Dembele et al., 1999). The mode of action of these insecticides is to phosphorylate or carbamilate AChE (Fleming, 1981). This results in the increase of acetylcholine levels at the nerve synapse causing continual stimulation of the fibers and eventual failure of nerves to repolarize (Dembele et al., 1999).

Data of the present study revealed dose-time dependence and organ specific of glyphosate formulation-induced inhibition of cholinesterase in *C. gariepinus* tissues with the maximal inhibition occurred on day 28th. The pesticide inhibited the activities of the enzyme in all the tissues examined. This could be attributed to a decrease in gene transcription, translation that enhances cholinergic activity, thereby improving cognitive function especially unconscious mental activities (Shahidi et al., 2008). The inhibition of AChE activities in brain, serum and liver of *C. gariepinus* in this investigation is in accordance with that recorded inbrain of *Cyprinus carpio* exposed to dimethoate (Satyadevan et al., 1993), in brain of *Oreochromis niloticus* exposed to malathion and bayluscide for 30 days (Danasoury et al., 1997), in brain and muscle of *Chinook salamon* exposed to chloropyrifos for 96 h (Wheelock et al., 2005), and in brain, serum and liver of *O. niloticus* exposed to malathion (1.75 mg/L),lannate (0.65 mg/L) and phenol (9 mg/L) (Nahed, 2006). These results also agreed with those of Kuhn and Streit (1994) on invertebrate.

High inhibition (83.8%) observed in brain tissues of *C. gariepinus* in this investigation could be detrimental to the fish health. Coppage et al. (1975) reported that inhibition of brain AChE in the range of 70–80% is critical to fishes, as it causes jerky movement in the fish treated with the enzyme inhibitor. Comparatively higher inhibition was also observed in brain tissues (95%) of monocrotophos exposed *Oreochromis mossambicus* (Venkateswara, 2004). Similar observation in AChE inhibition in fish tissues were reported in fish exposed to chlorpyrifos and profenofos (Jeyarathi Shanthi and Jebanesan, 2001; Kumar and Chapman, 1998; Venkateswara et al., 2003a,b).

The activities of the enzyme was overwhelmed by the inhibitor at a low dose of the *G. kola* seed's extract and tissue's responses differs, but improved as the concentrations of the extract increases. This showed that, the restorative mechanism of the plants extract is dose, time dependent and organ specific. Similar findings were reported when natural therapeutic agents were used to treat neurological disorder such as memory dysfunction and cognitive deficits with medicinal plants and nutrient supplement (Joseph et al.,1999; Shukitt-Hale et al., 2008; and Durairajan et al., 2008). These findings demonstrated that glyphosate formulation induces an inhibition of brain, liver and serum cholinesterase activities in the conscious fish and apparently fully reversible in *G. kola* seed's extract treatments. An indication that the plant seed extract is a reliable antidote for anticholinesterase agents.

5. Conclusion

This is the first study that assesses the antidotal prospect of *G. kola* on inhibited AChE. Herbs for acetylcholine work in several ways. Some adhere to acetylcholine receptors and stimulate the neurotransmitter, thus increasing its activity. Others inhibit AChE, which breaks down acetylcholine. By stopping the breakdown of acetylcholine, more of it is left between nerve cells, increasing its levels and duration of action. The enzyme catalyzes the hydrolysis of acetylcholine to choline and acetic acid. These are reabsorbed and used as raw materials for the continued acetylcholine productions. From this investigation, *G. kola* benefits may hold out Cholinesterase an important enzyme needed for the proper functioning of the nervous systems of humans, other vertebrates, and insects (Carlock et al., 1999). The plant's extracts normalize the secretion of acetylcholinesterase, so as to stabilize the concentrations of the neurotransmitter acetylcholine, for effective and efficient flows of signal. As more scientific studies are undertaken, it is possible that *G. kola* may be an important part of health maintenance and disease prevention that is prescribed by doctors in the future. Conventional therapies use drugs to increase acetylcholine for treating diseases such as glaucoma, Alzheimer's or myasthenia gravis. Further study is needed on the recovery potential of this plant seed's extract on other aquatic organisms and mammals, and on how plant that provide food for the aquatic organisms could be protected from glyphosate and other pesticides using plant products.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bjbas.2014.11.003

REFERENCES

Adaramoye OA, Farombi EO, Adeyemi EO, Emerole GO. Comparative study on the antiox- dant properties of flavonoids of *Garcinia kola* seeds. Pak J Med Sci 2005;21(3):331–9.

Braga M, Dal Sasso PC, Culici M, Bianchi T, Bordoni L, Marabini L. Anti-inflammatory activity of thymol: inhibitory effect on the release of human neutrophil elastase. Pharmacology 2006;77(3):130–6. http://dx.doi.org/10.1159/000093790. PMID 16763380.; Retrieved January 27, 2008.

Bocquene G, Bellanger C, Caudiou Y, Galgani F. Joint action of combination pollutants on the AChE activity of several marine species. Environ Toxicol Chem 1995;4:266–79.

Campbell D. A review of the culture of Sarotherodon melanotheron in West Africa. FAO Corporate Document Repository; 1978. Project RAF/82/009.

Carlock LL, Chen WL, Gordon EB, Killeen JC, Manley A, Meyer LS. Regulating and assessing risks of cholinesterase-inhibiting pesticides: divergent approaches and interpretations. J Toxicol Environ Health B 1999;2:105–60.

Coppage DL, Mathews E, Cook GH, Knight J. Brain AChE inhibition in fish as a diagnosis of environmental poisoning by malathion, O-o-dimethyl S-(1, 2 Dicarbethoxylthyl) hosphorodithioate. Pestic Biochem Physiol 1975:536–42.

Da-Yuan Z, Dong-Lu B, Xi-Can T. Recent studies on traditional Chinese medicinal plants. Drug Dev Res 1996;39:147–57.

Dai Q, Borenstein AR, Wu Y, Jackson JC, Larson EB. Fruit and vegetable juices and lzheimer's disease: the Kame Project. Am J Med 2006;119:751–9.

Danasoury MA, Reda LA, Shoukry A, Fayomy F. Biochemical target effected by two pollutants. J Egypt Ger Sco Zool 1997;22(A):33–54.

Dembele K, Haubruge E, Gaspar C. Recovery of Acetylchoinesterase activity in the common carp (*Cyprinus carpio* L) after inhibition by organophosphate and carbamate compound. Bull Environ Contam Toxicol 1999;62:731–42.

Durairajan SS, Yuan Q, Xie L, Chan WS, Kum WF, Koo I, et al. Salvianolic acid B inhibits Aβ fibril formation anddisaggregates preformed fibrils and protects against Aβ-induced cytotoxicty. Neurochem Int 2008;52:741–50.

Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88–95.

Ezemonye LIN, Ikpesu TO. Evaluation of sublethal effects of Endosulfan on cortisol secretion, Glutathione s-transferase and Acetylcholinesterase activities in *Clarias gariepinus*. Toxicol Ind Health 2012;29(4):325–33. http://tih.sagepub.com/content/29/4/325.

FEPA. Guidelines and standards for environmental pollution control in Nigeria, vol. 27. Nigeria: Federal Environmental Protection Agency; 1991. p. 20.

Finney DJ. Probit analysis. Cambridge,England: Cambridge University Press; 1971.

Fleming WJ. Recovery of cholinesterase activity in five avian species exposed to dicrotophos an organophosphorous pesticide. Pestic Biochem Physiol 1981;16:129–35.

Galloway TS, Handy RD. Immunotoxicity of organophosphorous pesticides. Ecotoxicology 2003;12:344–63.

Handy RD, Galloway TS, Depledge MH. A proposal for the use of biomarkers for the assessment of chronic pollution and in regulatory toxicology. Ecotoxicology 2003;12:331–43.

Haubruge E, Toutant JP. L'AChE du systeme olfatif de la carpe Cyprinus carpio L. (POISSON: CYPRINIDE): forme moleculaire et inhibiton in vivo et in vitro par le carbofuran. Belg J Zool 1997;127:63–73.

Heckel E, Schlagdenhauffen F. Some African kolas, in their botanical, chemical and therapeutical aspects. Am Pharm 1884;56:81–177.

Idodo UG. Fresh water fishes of Nigeria (Taxonomy, ecological notes, diet and utilization). Benin City, Nigeria: Idodo Umeh Publications; 2003. p. 250.

Ikpesu TO, Tongo I, Ariyo A. Restorative prospective of powdered seeds extract of *Garcinia kola* in *Chrysichthys furcatus* induced with *Glyphosate* formulation. Chin J Biol 2014. http://dx.doi.org/10.1155/2014/854157. ArticleID 854157.

Iwalewa EO, Adewumi CO, Omisore NOA, Adebanji OA, Azike CK, Adigun AO. Pro- and antioxidant effects and cytoprotective potentials of nine edible vegetables in Southwest Nigeria. J Med Food 2005;8(4):539–54.

Iwu MM. Biflavanones of Garcinia: pharmacological and biological activities. In: Cody V, Middleton E, Harbone JB, editors. Plant flavonoids in biology and medicine. New York: Alan R. Liss.; 1986. p. 485–8.

Iwu MM, Igboko OA, Onwuchekwa UA, Okunji CO. Evaluation of the antihepatotoxic activity of the biflavonoids of *Garcinia kola* seed. J Ethnopharmacol 1987;21(2):127–38.

Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, McEwen JJ. Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioural deficits with blueberry, spinach, or strawberry dietary supplementation. J Neurosci 1999;19:8114–21.

Jeyarathi Shanthi T, Jebanesan A. Effect of sub lethal concentration of chlorpyrifos on AChE activity in freshwater fish Cyprinus carpio communis (Linnaeus). Convergence 2001;3(1–4):31–5.
Kuhn K, Streit B. Detecting sublethal effect of organophosphates by measuring AChE activity in *Gammarus*. Bull Environ Contam Toxicol 1994;53:398–404.
Kumar A, Chapman JC. Profenofos toxicity to the eastern rainbow fish (Melanotaenia duboulayai). Environ Toxicol Chem 1998;17:1799–806.
Letenneur L, Proust-Lima C, Le Gouge A, Dartigues JF, Gateau P. Flavonoid intake and cognitive decline over a 10-year period. Am J Epidemiol 2007;165:1364–71.
Lopez S, Bastida J, Viladomat F, Codina C. Acetylcholinesterase inhibitory activity of some Amaryllidaceae alkaloids and Narcissus extracts. Life Sci 2002;71:2521–9.
Luke JH, Hill F, Dieter M. Cholinesterase (ChE) response and related mortality among birds fed ChE inhibitor. Arch Environ Contam Toxicol 1975;3:1–21.
Morris MC, Evans DA, Tangney CC, Bienias JL, Wilson RS. Associations of vegetable and fruit consumption with age-related cognitive change. Neurology 2006;67:1370–6.
Nahed SG. Biochemical responses in *Oreochromis niloticus* after exposure to sublethal concentrations of different pollutants. Egypt J Aquat Biol Fish 2006;10:181–93.
OECD (Organization for Economic Cooperation and Development). Guideline for the testing of chemicals: fish, acute toxicity test. 1992. No. 203.
OECD. OECD guideline for the testing of chemicals 407. Reapeted Dose 28-day Oral Toxicity Study in Rodent. 1997. Adopted on July 1995.
Onunkwo GC, Egeonu HC, Adikwu MU, Ojile JE, Olowosulu AK. Some physical properties of appendixtted seed of *Garcinia kola* (Heckel). Chem Pharm Bull 2004;52(6):649–53.
Oyelese OA, Faturoti EO. Growth and mortality estimates in *Clarias gariepinus* fed graded levels of processed cassava peels. J Trop For Resour 1995;11:71–81.
Rehman EU. Indigenous knowledge on medicinal plants, village Barali Kass and its allied areas, District Kotli Azad Jammu and Kashmir,Pakistan. Ethno Leafl 1995;10:254–64.
Reish DJ, Oshida PS. Manual of methods in aquatic environment research. Part 10. Short-term static bioassays. FAO Fisheries Technical Paper No.247. Rome: FAO; 1986.
Ryan TA, Joiner BL, Ryan BF. Minitab reference manual. Boston ,Massachusetts: Duxbury Press; 1981. 341 pp.
Satyadevan S, Kumar S, Tembhre M. Acetylcholinesterase activity and enzymes kinetics in the brain of common carp *Cyprinus carpio* subjected to sub-lethal exposure to dimethioate. J Biotechnol Biochem 1993;57(9):1566–77.
Shukitt-Hale B, Lau FC, Joseph JA. Berry fruit supplementation and the aging brain. J Agric Food Chem 2008;56:636–41.
SAS Institute, Inc. User guide: Statistics version 5 edition. Cary: North Carolina; 1985. 956 pp.
Shahidi S, Komaki A, Mahmoodi M, Atrvash N, Ghodrati M. Ascorbicacid supplementation could affect passive avoidance learning and memory. Brain Res Bull 2008;76:109–13.
Venkateswara RJ. Effects of monocrotophos and its analogs in AChE activity's inhibition and its pattern of recovery on euryhaline fish, *Oreochromis mossambicus*. Ecotoxicol Environ Saf 2004;59:217–22.
Venkateswara Rao J, Shipanjali D, Kavitha P, Madhavendra SS. Toxic effects of profenofos on tissue AChE and gill morphology in a euryhaline fish *Oreochromis mossambicus*. Arch Toxicol 2003a;77(4):227–32.
Venkateswara Rao J, Shoba Rani C, Kavitha P, Nageswara R, Madhavendra SS. Toxic effects of chloropyrifos on *Oreochromis mossambicus*. Bull Environ Contam Toxicol 2003b;70(5):985–92.
Walujo BP, Frederick AL. Parallel measurement of brain Acetylcholinestaerase and the muscarinic cholinergic receprtor in the diagnosis of acute, lethal poisoning by anti-cholinesterase pesticides. J Wild Life Dis 1991;27(1):110–5.
Weremczuk-Jezyna I, Kisiel W, Wysokińska H. Thymol derivatives from hairy roots of *Arnica montana*. Plant Cell Rep 2006;25(9):993–6. http://dx.doi.org/10.1007/s00299-006-0157-y. PMID 16586074.
Wheelock CE, Eder KJ, Wemer 1, Huanyg H, Jones PD, Brammell BF. Individual variability in esterase activity andCY T IA levels in Chinook salmon *Oncorhynchus tshawytscha* exposed to esfenvalerate and chloropyrifos. Aquat Toxicol 2005;4:172–92.
WHO. Guidelines for drinking water quality, vol. 1; 2006. Geneva.
Wiegand C, Pflugmacher S, Oberemm A, Meems N, Beattie KA, Steinberg CEW, et al. Uptake and effects of Microcystin – LR on detoxication enzymes of early life stages of the zebra fish (*Danio rerio*). Environ Toxicol 1999;14:89–95.